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EFFECTS OF PERCHLORATE ON THYROIDAL UPTAKE OF IODIDE WITH CORRESPONDING HORMONAL CHANGES

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TECHNICAL REVIEW AND APPROVAL

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

David R. Mattie

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Perchlorate interferes with iodide accumulation in the thyroid resulting in reduced thyroidal hormone synthesis. The objective of this project was to conduct a series of experiments to investigate the role of perchlorate on the uptake of iodide in the thyroid and its effects on the synthesis of thyroid hormones. A time course study with perchlorate (single iv dose) was performed to investigate its kinetic behavior in the adult male rat. Rats were injected with 0, 0.01, 0.1 1 and 3 mg perchlorate/kg via tail vein, challenged with 125I with carrier at 2 h post dosing, and euthanized at predetermined time points. Three groups (n=6 per group) of 1-, 5-, and 14-day drinking water studies were performed at different levels of perchlorate (0, 1, 3 and 10 mg/kg). At the end of exposure, rats were challenged once by tail vein injection with 125I with carrier. Dose-related inhibition of thyroid iodide uptake after a single iv dose of perchlorate, and both dose- and time-dependent changes in inhibition of thyroid iodide uptake were observed in drinking water studies. In both the single dose and drinking water studies, TSH and free T4 levels were elevated with T4 levels decreased. Serum T3 levels in drinking water studies remained unchanged in all dosing groups.

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PREFACE

This study was conducted at the Air Force Research Laboratory, Human Effectiveness Directorate, Operational Toxicology Branch (AFRL/HEST). Work was performed under the following contracts and supervision: ManTech GEOCENTERS Joint Venture (F41624-96-C-9010, Program Manager: Dr. Darol Dodd, P.O. Box 31009, Dayton, Ohio 45437) and Operational Technologies Corporation (DAHA 90-06-D-0014, Manager: Dr. Peter Lurker, 1370 N. Fairfield Rd., Suite A, Beavercreek, Ohio 45432). Mr. Chuck Goodyear is a statistical consultant for AFRL, Human Effectiveness Directorate, Crew Systems Interface Division (AFRL/HEC), Wright-Patterson Air Force Base, Ohio.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

LIST OF ABBREVIATIONS AND ACRONYMS

μg	microgram
μL	microliter
μm	micrometer
γ	gamma
^{125}I	carrier-free iodide
AP	ammonium perchlorate
ClO_4^-	perchlorate anion
G	gravity
h	hour
HPLC	high pressure liquid chromatography
iv	intravenous
kg	kilogram
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mM	milli-molar
n	sample size
NIS	sodium iodide symporter
RIA	radioimmunoassay
rpm	rotations per minute
SD	standard deviation
T_3	triiodothyronine
T_4	tetraiodothyronine or thyroxine
TBG	thyroxine binding globulin
TCA	trichloroacetic acid
Tg	thyroglobulin
TSH	thyroid stimulating hormone

EFFECTS OF PERCHLORATE ON THYROID UPTAKE OF IODIDE WITH CORRESPONDING HORMONAL CHANGES

INTRODUCTION

The perchlorate anion, ClO_4^-, acts as a competitive inhibitor of iodide uptake in the thyroid gland (Wolff, 1998). Its molecular size and charge are similar to iodide, an essential constituent for the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). The Na^+/I^- symporter (NIS), a protein channel located in the basolateral membrane of the thyroid follicular cells, actively transports iodide (Carrasco, 1993; Eskandari *et al.*, 1997). Iodide enters the follicular cells against an electrochemical gradient by a carrier-mediated mechanism driven by ATP (adenosine triphosphate). Perchlorate competes with iodide for uptake at this iodide-concentrating step (Goldman and Stanbury, 1973), potentially leading to hypothyroidism. As a result perchlorate has been used to treat hyperthyroidism (Wolff, 1998).

In the thyroid follicular cell, iodide passively diffuses across the apical membrane into the colloid, where inorganic iodide is oxidized to molecular iodine by thyroid peroxidase, a membrane-bound enzyme associated with the apical membrane (Nunez and Pommier, 1982). Iodination of tyrosyl residues of thyroglobulin (Tg) takes place in the colloid (called the organification of iodine) and coupling of iodotyrosine with Tg forms T_4 and T_3. Thyroid hormones (T_4 and T_3) bound to Tg is the transported from the colloid into the follicle (Carraso, 1993). Although other tissues such as salivary gland, choroid plexus, gastric mucosa and mammary gland have iodide concentrating ability, synthesis of thyroid hormones takes place only in the thyroid gland (Wolff, 1998).

Proteolysis of Tg results in release of iodothyronines into the blood. T_4 is the primary iodothyronine product of a normal thyroid and is converted to T_3 by the enzyme 5'-deiodonase, located mainly in the peripheral tissues (Greenspan, 1994; Taurog, 1991; Wolff, 1998). Excess iodide in the circulating blood prevents secretion of thyroid hormone and autoregulation of iodide maintains its homeostatic level in plasma (Wolff and Chaikoff, 1948). Circulating T_3 is produced primarily from peripheral deiodination of T_4 in liver, kidney, thyroid and pituitary (type I enzyme), and in other tissues such as skin, cerebral cortex and placenta (type II enzyme). Type I enzyme produces active T_3, while type II deiodinase generates biologically inactive reverse T_3. In addition, thyroxine is metabolized in liver by sulfation and glucuronidation of the phenolic hydroxyl group (Visser, 1994).

Thyroid stimulating hormone (TSH) produced in the pituitary gland influences iodide transport and regulates biosynthesis of thyroid hormones (Halmi, 1961). TSH stimulates the uptake of iodide by sodium iodide symporters and the organification and secretion of thyroid hormones (T_4 and T_3). This process is inhibited by excess iodide (Cavalieri, 1997). In adult rat studies (Caldwell *et al.*, 1995; Fisher *et al.*, 2000), ammonium perchlorate (AP) inhibited the uptake of iodide into the thyroid and led to increased serum TSH in response to decreasing circulating levels T_3 and T_4. The thyroid gland can compensate by TSH-stimulated increase in uptake of

iodide. However, chronic stimulation by TSH can result in increased thyroid weight, goiter and hypothyroidism (Fukuda *et al.*, 1975; Gerber *et al.*, 1981).

The objective of this study was to investigate the temporal relationships between administered perchlorate dose, concentration of circulating perchlorate in serum, and inhibition of the uptake of iodide in the thyroid gland. Inhibition of uptake of iodide in the thyroid was examined in rats dosed intravenously with perchlorate or given drinking water treated with perchlorate. A portion of this work was reported elsewhere (Fisher *et al.*, 2000).

METHODS

Chemicals

Ammonium perchlorate (99.8%), sodium iodide (99.5%), sodium hydroxide (99.99%) and trichloroacetic acid (98%) were purchased from Aldrich (Milwaukee, WI). Carrier-free ^{125}I (16.0mCi/μg) was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Water used in this study was treated by a reverse osmosis system and then deionized.

Animals

Male Sprague Dawley rats (310 ± 30g) used throughout the experiments were obtained from Charles River Laboratories, Wilmington, MA. They were kept in separate cages and allowed access to commercial rat chow (Formulab, 5008) and water *ad libitum*. Rats used for urine collection were individually placed in metabolism cages; water and powdered food were freely available. Six rats were used per group in the kinetic studies and, for the thyroid hormone studies, eight rats were used per group.

Unless mentioned otherwise, intravenous dosing was carried out via lateral tail vein injection (volume = 0.67 mL/kg) of carrier-free ^{125}I and non-radiolabeled iodide (carrier) mixed in physiological saline, providing a total concentration of 33 μg iodide/kg. Controls received 0.67 mL/kg of physiological saline. Schedules for *iv* administration and euthanization of rats by CO_2 asphyxiation were always in the morning (5:30-11:30 am) to minimize the influence of diurnal variation on thyroid hormone levels in serum.

Animal Studies

For the following studies, the term total iodine includes "bound iodine plus free iodide"; free iodide refers to "free inorganic iodide". ^{125}I is referred to as "carrier-free iodide" unless otherwise called "^{125}I with carrier", which refers to ^{125}I plus non-radiolabeled iodide.

Intravenous Dosing Studies

^{125}I and ^{125}I with Carrier: Rats (n = 6 per time point) were administered a single *iv* tail vein injection once with physiological saline (control group) or 33 μg/kg ^{125}I (with carrier) in physiological saline. They were euthanized by CO_2 asphyxiation at 5, 15 and 30 minutes (min), 1, 2, 6, 9 and 24 hours (h) post dosing to collect thyroid and blood from the vena cava. Rats for the 24 h time point were placed individually in metabolism cages for up to 96 h to collect urine. Another group of rats (n=6 per time point) were injected with 2.6 ng/kg of ^{125}I in physiological saline and euthanized at 5, 15 and 30 min, 1, 2 and 6 h post dosing. Thyroid and blood were harvested.

Perchlorate with ^{125}I (with Carrier) Challenge: Rats (n=6 per dose and per time point) were injected with one of five doses of perchlorate (0.0, 0.01, 0.1, 1.0 and 3.0 mg/kg). At 2 h post dosing, they were challenged with ^{125}I with carrier (33 μg/kg) by intravenous injection and euthanized at 5, 15 and 30 min, 1, 2, 6, 9 and 24 h post dosing of iodide. This corresponds to 2.08, 2.25, 2.5, 3, 4, 8, 11 and 26 h, respectively, after dosing with perchlorate. Blood and thyroid were harvested from all time point groups; urine was collected from rats in the 24 h dose group. Perchlorate and iodide levels were determined in the thyroid, serum and urine.

Perchlorate with Thyroid Hormone Profile: The highest *iv* dose of perchlorate (3 mg/kg) was chosen for a single *iv* dosing study to investigate the temporal relationship between inhibition of iodide uptake in the thyroid and biosynthesis of TSH and thyroid hormones. Rats (n=8 per dose and per time point) were injected with either perchlorate (3 mg/kg) or saline (control) and euthanized at 8, 12, 24 and 48 h post dosing. Perchlorate, TSH and thyroid hormones were measured in serum.

Drinking Water Studies

Perchlorate with ^{125}I (with Carrier) Challenge: To investigate the inhibitory effects of perchlorate, three drinking water studies (1, 5 and 14 days) were performed at different levels of perchlorate with target concentrations of 0.0, 1.0, 3.0 and 10.0 mg/kg-day continually exposed via drinking water. At the end of day 1, 5 or 14, rats (n=6 per group) were challenged once with 33 μg/kg ^{125}I with carrier and euthanized at 2 h post iodide dosing. Blood and thyroid gland were collected for perchlorate and iodide analyses in serum.

Perchlorate with Thyroid Hormone Profile: Three groups of rats (n=8) were continually exposed via drinking water at different levels of perchlorate for 1, 5 and 14 days with target concentrations of 0.0, 0.1, 1.0, 3.0, and 10.0 mg/kg-day. They were euthanized at the end of the experiments and blood was collected for analysis of thyroid hormones and TSH levels in serum.

Analytical Procedures

^{125}I with Carrier Analysis in Tissues

Thyroid lobes were weighed and one or two lobes were placed in a 0.2 mL-micro tissue homogenizer (Kontes Glass Co., Vineland, NJ) and homogenized with 100 μL of water. Thyroid homogenate was transferred into a polystyrene, round bottom tube (12 m x 75 mm). The micro tissue homogenizer was washed 3 times with 120 μL of water to ensure complete transfer of the homogenate. Total radioactivity of the thyroid homogenate was counted by a γ-counter (Packard Instrument Co., Meriden, CT). Half a milliliter of 10% trichloroacetic acid (TCA) was then added to precipitate protein. The homogenate was vortexed for 2 to 3 seconds, centrifuged for 10 min at 5000 rpm, and the supernatant was transferred into a polystyrene tube. Half a milliliter of 5% TCA was added to the pellet. The pellet was gently dislodged and shaken. The mixture was centrifuged and decanted as above. Using half a milliliter of 2.5% TCA, the centrifuge

procedure was again repeated. Radioactivity of the supernatant (free) and the pellet (bound) was measured by a γ-counter.

Aliquots of serum or urine were placed into a round polystyrene tube and total radioactivity was determined by a γ-counter. Free and bound radioactivity measurements of serum or urine was carried out in the same manner as described for thyroid homogenate.

High Pressure Liquid Chromatography (HPLC) Analysis of Perchlorate: Thyroid specimens from control and perchlorate dosed rats were homogenized in 250 µL of deionized water in a micro tissue homogenizer. Homogenates were centrifuged at 31,500 x G for 30 min at 4°C. The supernatants were diluted 10, 25, 50 and 100 times with water bringing the final volume to 2 mL. The diluted supernatants were filtered through 0.45 µm non-sterile acrodisc syringe filters provided with versapor (supported acrylic copolymer) membranes (Pall Gelmann Laboratory, Ann Arbor, MI). One mL of each filtrate was injected into the HPLC system using an autosampler.

Perchlorate analyses of serum and urine samples were carried out by mixing 100 µL samples with 300 µL ice-cold ethanol. The mixture was centrifuged at 31,500 x G for 30 min at 4°C. Supernatants were evaporated to dryness under nitrogen at 37°C and reconstituted in 2 mL of water. Reconstituted serum and urine samples were filtered as above for thyroid samples. One mL of each filtrate was injected into the HPLC.

HPLC Conditions: Analyses were performed with a Model Dx-300 liquid chromatographic system equipped with a background conductivity suppressor (Dionex, Sunnyvale, CA). The system consists of an advanced gradient pump (AGP standard size), conductivity detector (CDM-3), an anion self regenerating suppressor (ASRS 4mm) for the reduction of the background conductivity of the eluent, an autosampler (AS-3500), a 1000 µL sample loop, a computer interface (ACI), and software (Autolon 450). Separation by isocratic elution of perchlorate was performed on a Dionex AS11 analytical column (4 x 250 mm) preceded by a Dionex AG 11 guard column (4 x 50 mm). The sensitivity of the detector was maintained between 0.5 and 100 µs depending on the concentration. The mobile phase (flow rate = 1 mL) was 100 mM sodium hydroxide in water. The mobile phase was filtered through a 0.45 µm filter provided with a nylon membrane (Micron Separations Inc., Westboro, MA), and degassed under vacuum before use. Perchlorate standards were prepared in water in the range of 0.5 to 100 ng/mL.

Analysis of TSH and Thyroid Hormones: The hormone assays were conducted using radio-immunoassay (RIA) kits following the manufacturers' instructions. Kits for T_4, free T_4 and T_3 were purchased from Diagnostic Product Corp. (Los Angeles, CA) and kits for TSH from Amersham Corp. (Arlington Heights, IL). Standards and samples were run in triplicate and assay kits from the same batch number and with the same expiration date were used for all thyroid hormones or TSH measurements for each animal. Tracer ^{125}I activity was measured with a γ-counter.

Batch numbers and expiration dates for the kits are as follows.

- Total T_4 kits (T_4 calibrators batch # CT43, expired Dec 31, 2000; ^{125}I T_4 batch # TT42, expired July 31, 1999; T_4-coated tubes batch # TT41, expired Jan 31, 2000)
- Free T_4 kits (free T_4 calibrators batch # F4C-8, expired Oct 31, 2000; ^{125}I free T_4 batch # TF42; expired July 31, 1999; free T_4 Ab-coated tubes batch # Tf41 Feb 29, 2000)
- Canine T_3 kits (canine T_3 calibrators batch # C3D3-8, expired July 31, 1999; ^{125}I canine T_3 batch #, TC32, expired July 31, 1999; canine T_3 Ab-coated tubes batch # TC31, expired Oct 31, 1999)
- TSH kits (TSH assay system code RPA554, batch # 104A, expired 23 Jul 1999)

Statistical Analysis

In single dose *iv* studies, two-tailed two-sample t-tests were used to compare the control group and dose group. In the studies with multiple dose groups, a one-way analysis of variance was performed to determine whether there was a significant difference among groups. All paired tests among groups used two-tailed two-sample t-tests. All statistical comparisons utilized a minimum significance level of $p<0.05$.

RESULTS

Intravenous Administration of Iodide and Perchlorate

The highest concentration of plasma iodide observed in rats dosed with 33 μg/kg ^{125}I (with carrier) was 5.5-6 μg/100 mL serum. This dose was selected to investigate iodide kinetics in male rats. The time course for the ^{125}I (2.6 ng/kg) and for 33 μg/kg ^{125}I (with carrier) in the thyroid produced similar results for uptake in the thyroid. Figure 1 shows the percentage of bound iodide in the thyroids of rats dosed with ^{125}I (2.6 ng/kg) as compared to the thyroids of rats dosed with ^{125}I plus carrier (33μg/kg). Iodide uptake into the thyroid gland was very rapid and variable among individual rats during the first five minutes after dosing. At 1 h, about 90% of the administered dose was bound in the thyroid for both dose groups.

The time courses for perchlorate concentrations in the thyroid and serum are shown in Figures 2 and 3, respectively. At 2 hours and 5 minutes post dosing, thyroid concentrations of perchlorate at 0.01, 0.1, 1.0 and 3.0 mg/kg were 0.3, 2.64, 20.26 and 23.28 μg/g thyroid, respectively. Table 1 shows estimated half-lives of terminal phases for perchlorate in the thyroid and serum. As measured by bound ^{125}I, perchlorate-induced inhibition of ^{125}I uptake in the thyroid was 13, 24, 70 and 88% at 2 h and 11, 29, 55 and 82% at 9 h after dosing with ^{125}I with carrier for the 0.01, 0.1, 1.0 and 3.0 mg/kg dose groups, respectively (Table 2).

Mean urinary excretion (± SD) of perchlorate over a 24 h period was about 97% (± 2), 72% (± 1), 87% (± 17) and 91%(± 13) of the administered *iv* dose for the 0.01, 0.1, 1.0 and 3.0 mg/kg dose groups, respectively.

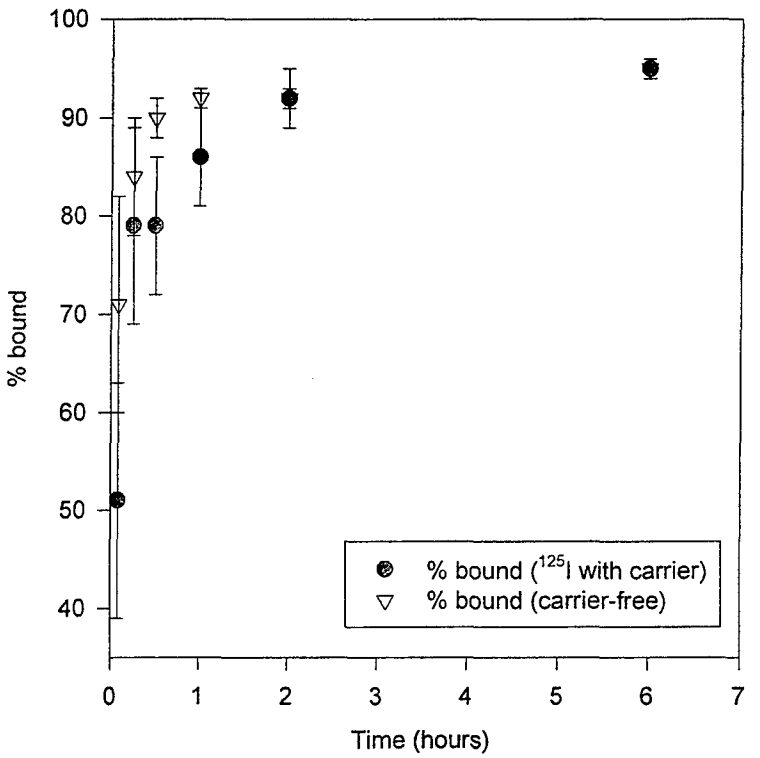

Figure 1. Percentages of bound iodine in the thyroid of male rats dosed with ^{125}I (carrier-free) and ^{125}I with carrier. Data are mean ± SD (n=6).

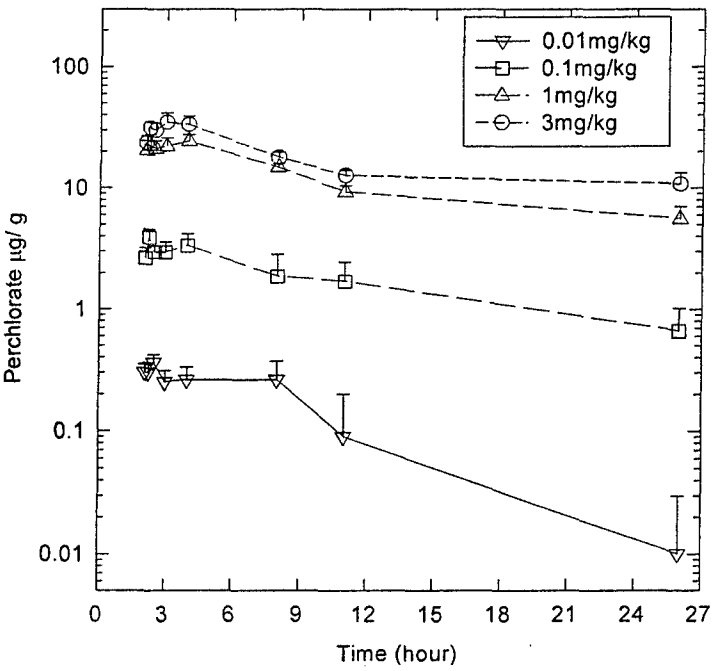

Figure 2. Perhclorate levels in thyroid of male rats after single *iv* injection of perchlorate. Data are mean ± SD (n=6).

TABLE 1. HALF-LIVES (HOURS) OF TERMINAL PHASES OF PERCHLORATE IN THE THYROID AND SERUM

Perchlorate Dose (mg/kg)	Thyroid	Serum
0.01	9	12
0.1	27	20
1.0	33	26
3.0	71	16

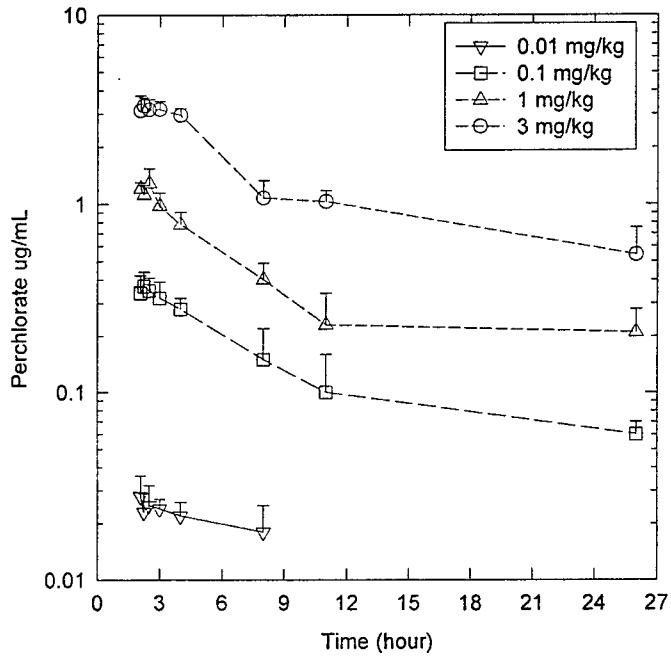

Figure 3. Perchlorate levels in serum of male rats after single *iv* injection of perchlorate. Data are mean ± SD (n=6).

TABLE 2. PERCENT INHIBITION OF IODIDE UPTAKE IN THE THYROID GLAND OF MALE RATS (N=6) DOSED WITH PERCHLORATE

Time Points	Perchlorate Dose (mg/kg)	Mean [Iodide] (μg/g)	% of Inhibition*
2 h	Control**	24.4	-
	0.01	21.3	13
	0.1	18.6	24
	1+	7.4	70
	3+	3.0	88
6 h	Control**	46.5	-
	0.01	36.7	21
	0.1	32.0	31
	1+	19.2	59
	3+	9.1	80
9 h	Control**	55.0	-
	0.01	49.2	11
	0.1+	39.2	29
	1+	24.7	55
	3+	10.0	82

* Percent of inhibition = (control mean – dose mean)*100/(control mean)

**Dosed with ^{125}I with carrier only (33 μg/kg)

+ Significantly different from control at $p<0.05$

Figure 4 depicts the inhibition time course of iodide uptake into the thyroid gland by perchlorate. The most pronounced inhibitory effects were at the 1 and 3 mg/kg perchlorate dose groups. By 24 h after dosing with ^{125}I (26 hours after dosing with AP), the inhibitory effects of perchlorate on ^{125}I uptake in the thyroid were still observed in the 1.0 and 3.0 mg/kg perchlorate dose groups (43 and 69%, respectively).

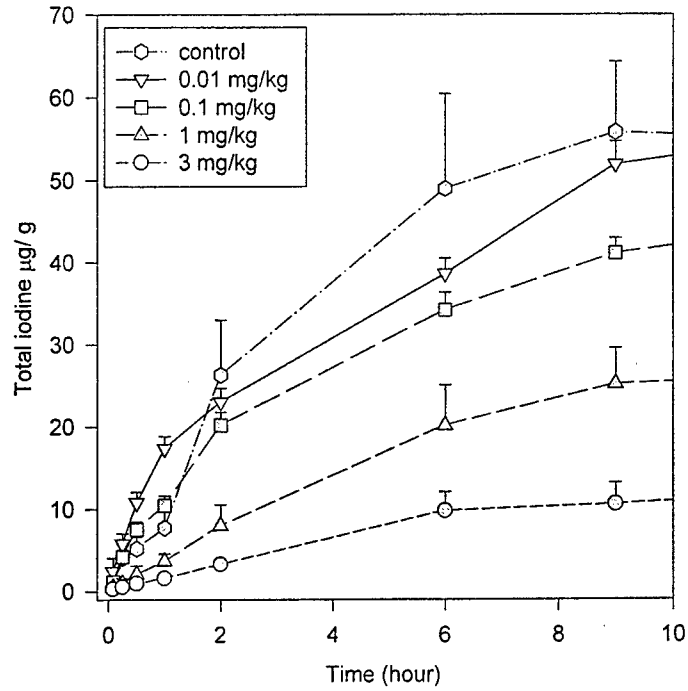

Figure 4. Total iodine levels in the thyroid of male rats after a single *iv* injection of perchlorate followed by an *iv* injection of ^{125}I with carrier at 2 hours later. Data are mean ± SD (n=6).

Figure 5 shows a time course of free iodide levels in the serum of rats injected intravenously with perchlorate. Since iodide levels of control, 0.01, 0.1, 1.0 and 3.0 mg/kg group were similar, only the control and 3 mg/kg dose groups are shown. The major route of elimination of iodide is urinary excretion (Perlman *et al.*, 1941). In our study, the control ^{125}I (with carrier) dosed rats excreted 89.5% (± 5.5) of their ^{125}I dose over a 24 hour period (mean±(SD)). The 0.01, 0.1, 1.0 and 3.0 mg/kg perchlorate dose groups excreted 87% (± 7.8), 86% (± 4.5), 88% (± 0), and 84% (± 7) of the ^{125}I dose in urine, respectively, over a 24 hour period (mean (± SD)). No bound iodide was detected in urine of rats dosed with ^{125}I (with carrier).

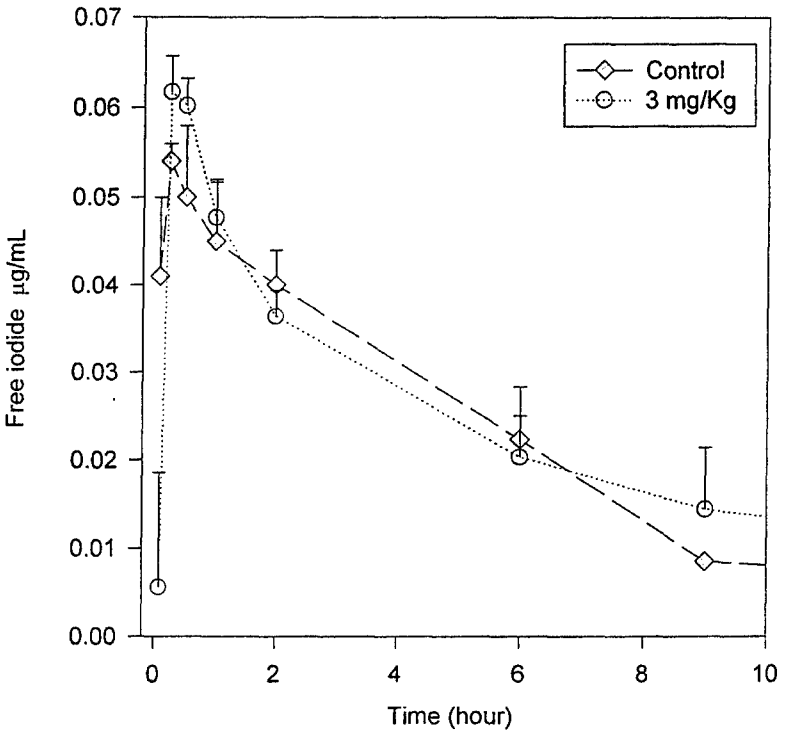

Figure 5. Free iodide levels in serum of male rats after a single *iv* injection of perchlorate followed by *iv* injection of ^{125}I with carrier two hours later. Data are mean ± SD (n=6).

Serum TSH levels increased 50 to 60% above controls by 12 hours and remained elevated until termination of the study (48 hours) while Serum T_4 concentrations decreased by about 20 to 30% from 12 to 24 hours (Fig. 6). T_3 levels returned to normal by 24 h post dosing. T_4 and free T_4 levels returned to normal range by 48 h post dosing. Thyroid hormone levels returned to control values and serum levels of TSH remained elevated at 48 h.

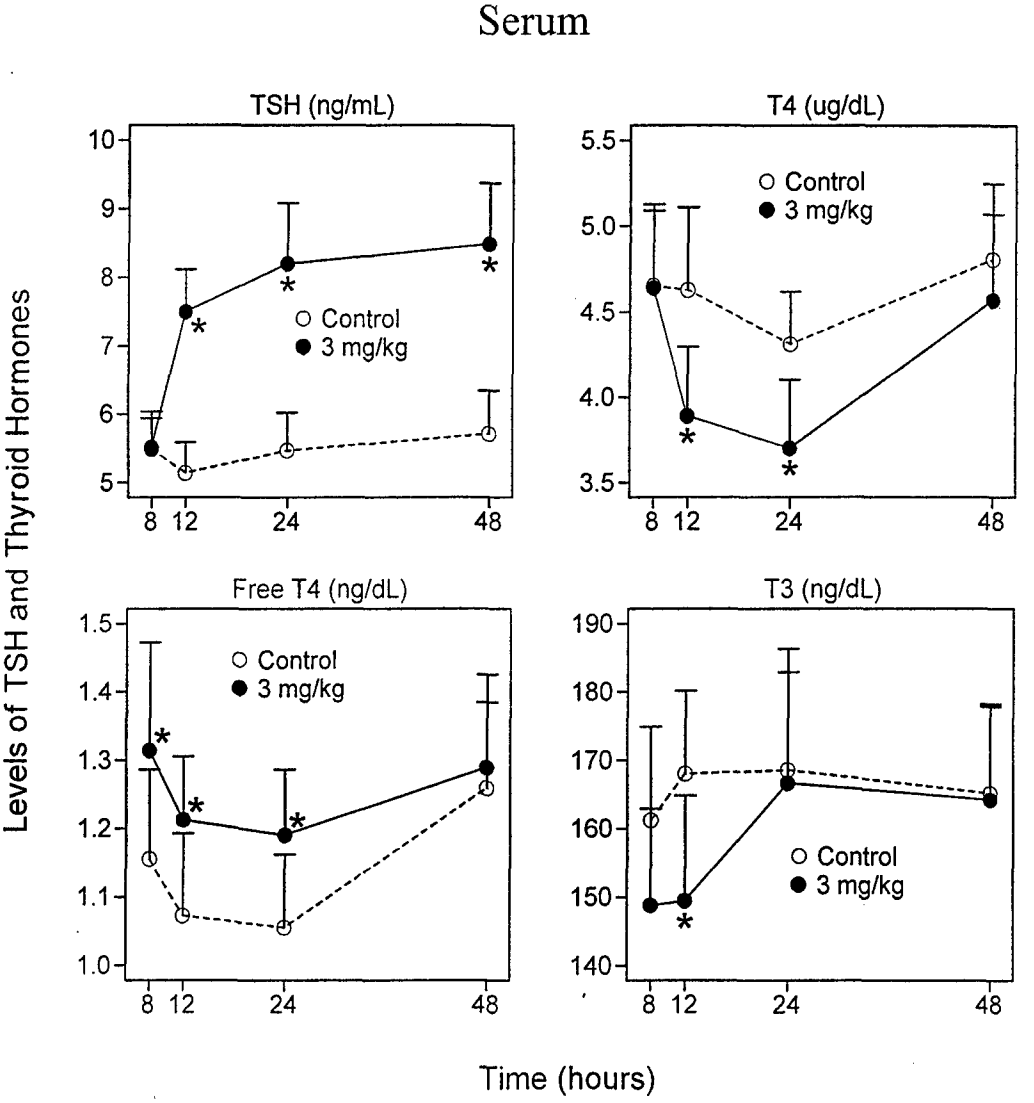

Figure 6. Levels of serum TSH and thyroid hormones of rats after *iv* injection of perchlorate (3mg/kg). Data are mean ± SD (n=8).
***Significantly different from control at p<0.05.**

Perchlorate drinking water studies

In the perchlorate drinking water studies, concentrations of free iodide in serum of rats were similar (0.03 to 0.058 μg/mL) to those seen in the single ^{125}I (with carrier) dosing study mentioned above. Inhibition of iodide uptake into thyroid by perchlorate is shown in Figure 7. There was a dose-related inhibition in the one-day treated group. The longer the rats were exposed to AP (i.e., 5 to 14 days), the fewer the inhibitory effects were observed. The thyroid gland appears to accommodate to longer-term perchlorate exposure, leading to no inhibitory effects at the end of the two-week drinking water study (except in the 10 mg/kg-day dose group).

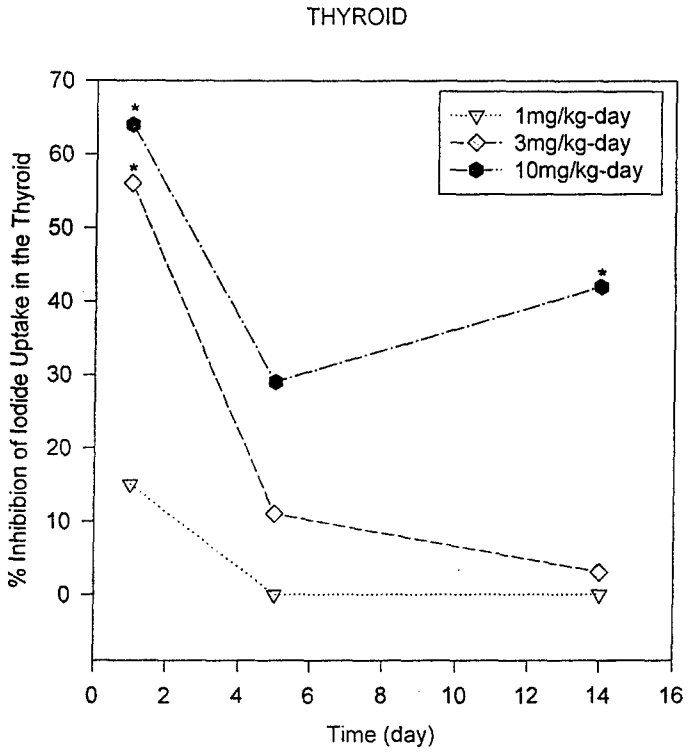

Figure 7. Percent inhibition of iodide uptake in the thyroid relative to control. Rats were provided perchlorate for 1, 5 or 14 days, and then challenged with ^{125}I with carrier. Data are mean values (n=6). *Significantly different from control at p<0.05.

Figure 8 depicts hormonal changes during perchlorate exposure in drinking water. In all treated groups, regardless of dose or the length of exposure time, TSH levels were increased. The serum T_4 levels initially decreased in all dose groups except the lowest (0.01mg/kg-day); by 14 days, the 0.1 and 1.0 mg/kg-day dose groups returned to control T_4 values. On day one, free T_4 levels increased in all dose groups; by day five they returned to control levels. By day 14, however, free T_4 levels in all dose groups were elevated, except in the 0.1mg/kg-day group.

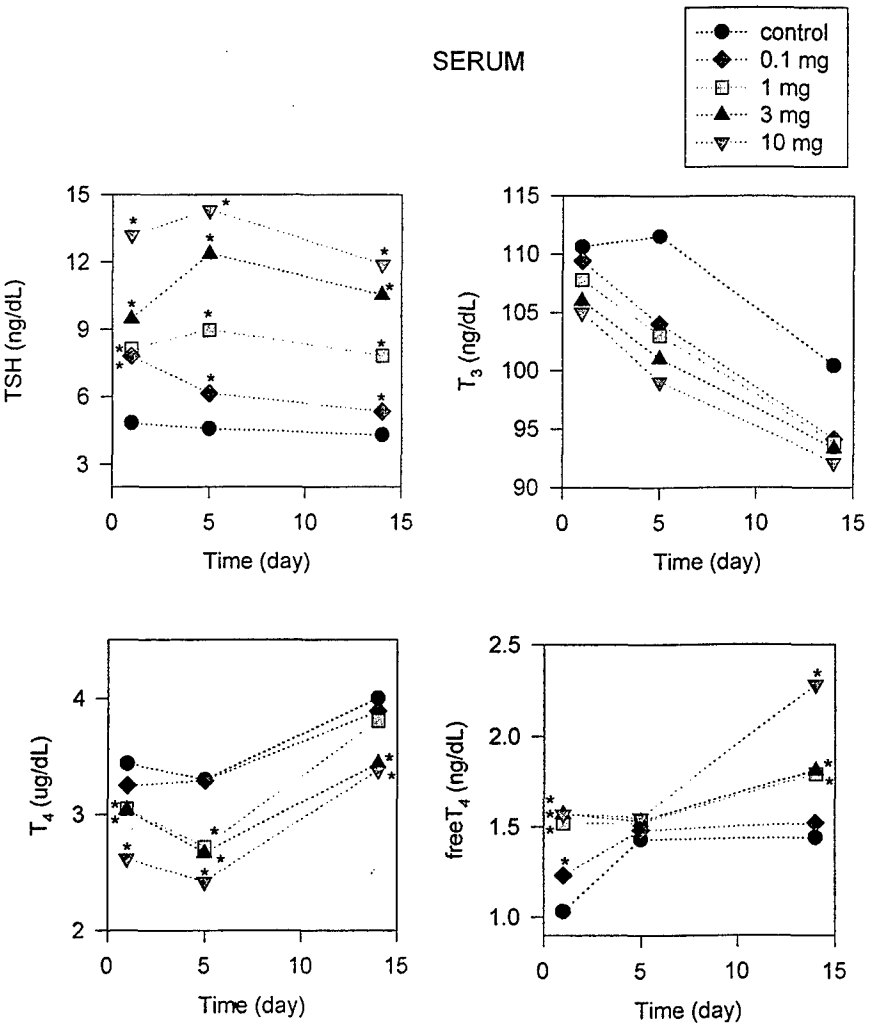

Figure 8. Hormonal changes by perchlorate in repeated doses at 0, 0.1, 1, 3 and 10 mg/kg-day for 1, 5 and 14 days exposure. Data are mean values (n=8). *Significantly different from control at $p<0.05$.

DISCUSSION

Tissue dosimetry of ^{125}I with carrier and perchlorate is essential to understand the effect of perchlorate on iodide uptake into thyroid. Target concentrations of ^{125}I with carrier and perchlorate in tissues, not administered doses, are needed to develop a pharmacokinetic model. Several kinetic studies of these compounds have been reported in the literature. However, the majority of these are reported as a ratio of tissue (e.g., thyroid) versus plasma concentration of radiolabeled iodide and/or perchlorate (Chow *et al.*, 1969; Chow and Woodbury, 1970; Wollman and Reed, 1959).

The thyroid gland has the ability to regulate uptake of excessive levels of iodide in the blood (Wolff-Chaikoff effect), which is one of the factors other than TSH that control iodide transfer into thyroid (Galton and Pitt-Rivers, 1959; Halmi *et al.*, 1956; Wolff, 1964). Wolff and Chaikoff (1948) reported that no autoregulation was observed when rats were dosed with 50 μg/kg iodide, while doses of 250 μg/kg iodide and higher resulted in autoregulation. The inhibition was correlated with plasma iodide concentration and the effective values were found to be above 20 to 35 μg/100mL. Our dose of 33 μg/kg of ^{125}I with carrier did not appear to cause the Wolff-Chaikoff effect.

Short-term perturbations of thyroid iodine economy by perchlorate and corresponding hormonal changes were investigated. Perchlorate is actively taken up by the thyroid gland and prevents iodide uptake into the thyroid, disrupting thyroid hormone synthesis and secretion (Anbar *et al.*, 1959; Capen 1996; Chow *et al.*, 1969; Wolff, 1998). Since perchlorate competitively inhibits iodide uptake into the thyroid, less iodide is available for organification. This results in lower concentrations of bound iodide in the thyroid gland thus lowering circulating levels of thyroid hormones. Reduction of circulating serum T_4 levels signals the pituitary gland to produce more TSH (D'Angelo *et al.*, 1976). TSH increases TSH receptors in the thyroid gland and activates the thyroid. The half-life of plasma T_4 is shorter (12 to 24 hours in rats) as compared to human T_4 (5 to 9 days) due to the different binding proteins (Vranckx *et al.*, 1994). In humans, circulating T_4 is bound to thyroxine-binding globulin (TBG) which is virtually undetectable in rats after eight weeks of age (Savu *et al.*, 1987; Vranckx *et al.*, 1994). In rats, T_4 binds predominantly to albumin and its binding affinity is far less than TBG (approximately 1000 times lower); (Capen, 1996). Differences in transport proteins and their affinity to the thyroid hormones result in different half-lives for thyroid hormone in the rat and human. In the rat, serum concentrations of circulating thyroid hormones after perchlorate exposure can be quickly depleted. This results in increased production of TSH. Therefore, the rat thyroid has a greater potential to develop hyperplasia.

Our data revealed that free T_4 and TSH levels increased in both single *iv* dose and drinking water studies. Perchlorate may displace thyroxine when it is bound to serum proteins resulting in elevation of free thyroxine levels (Yamada, 1967; Wolff, 1998). This may explain why free T_4 levels increased in the presence of perchlorate.

In the drinking water studies, the shorter the exposure period, the greater the percent inhibition of iodide uptake. By two weeks, there was no inhibition except at the 10 mg/kg-day dose. Levels of bound iodide in controls and dosed rats, except the highest dose group, were similar after two

weeks of AP exposure. Perchlorate-induced inhibitory effects of iodide uptake in the thyroid were dose- and time-dependent.

T_4 levels in the drinking water studies significantly decreased at most of the time points and TSH levels increased during the same time. Perturbation of thyroid hormone synthesis by perchlorate caused decreased T_4 levels, which resulted in increased TSH (negative feedback) and free T_4 in our studies.

We report dose-related inhibition of thyroid iodide uptake after a single *iv* dose of perchlorate, and both dose- and time- dependent changes in inhibition of thyroid iodide uptake in perchlorate drinking water studies. Urinary excretion was the major route of elimination of iodide and perchlorate. In both the single dose and drinking water studies, TSH and free T_4 serum levels were elevated while T_4 serum levels decreased. Serum T_3 levels in the drinking water studies remained unchanged in all dosing groups. These data demonstrate that upregulation of the thyroid is rapid and can compensate for the inhibitory effects of perchlorate.

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